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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/071,468	02/08/2002	Stephen J. Benkovic	P05537US1	9968

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EXAMINER
SWOPE, SHERIDAN

ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 09/23/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/071,468	BENKOVIC ET AL.	
	Examiner	Art Unit	
	Sheridan L. Swope	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

P riod for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *20 July 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) *8-13* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-7* is/are rejected.

7)☒ Claim(s) *6* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *8.14.03* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

Applicant's election with traverse of Invention I, Claims 1-7 in Paper No. 5 is acknowledged. The traversal is on the ground(s) that no undue burden would be imposed by examination of Groups I-III, since the inventions of Groups I-III are related with each group sharing a sequence encoding a substrate of a protease and changes in quenching of fluorescence being detected. This is not found persuasive Groups I-III are distinct for the reasons described in the prior action. As indicated by differing classifications for Groups I-III, a search for one group would not encompass a search for any other group and searching all groups would represent a burden on the Office. The requirement is still deemed proper and is therefore made FINAL.

Claims 8-13 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to nonelected Inventions, there being no allowable generic or linking claim. Claims 1-7 are examined on their merits.

Specification-Objections

Figure legend 4 is objected to. The legend describes 4A and 4B as being the same, but the data and the specification indicate that 4A and 4B are different.

Claims-Objections

Claim 6 is objected to for having "of" on line 7 in the phrase "expressing of the recombinant". Correction is required.

Claim Rejections - 35 USC § 112-Second Paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-7 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for the following reasons.

Regarding the phrase "carboxy terminal portion of a fluorescent reporter" on line 4, Claims 1 and 6 have two interpretations. Said carboxy terminal portion could be derived from the same fluorescent reporter having the "amino terminal portion" as recited in line 2 of Claims 1 and 6. Alternatively, the N- and C-terminal portions could be derived from either the same or different fluorescent reporters. Claims 2-5 and 7, as dependent from Claims 1 and 6, respectively, are rejected for the same reasons. For purposes of examination, it is assumed that the N- and C-terminal portions are derived from the same fluorescent reporter. It is suggested that applicants amend the "a fluorescent" on line 4 to "the fluorescent".

Regarding the phrase "expressing a recombinant fluorescent substrate" on line 6, Claim 1 has two interpretations. Said recombinant fluorescent substrate could be the fusion protein described in lines 1-5 of Claim 1. Alternatively, said recombinant fluorescent substrate could encompass any recombinant fluorescent substrate, including the fusion protein described in lines 1-5 of Claim 1. Claims 2-5, as dependent from Claim 1, are rejected for the same reasons. For purposes of examination, it is assumed that the recombinant fluorescent substrate is the fusion protein described in lines 1-5 of Claim 1. It is suggested that applicants amend the "a recombinant" on line 6 to "the recombinant".

In Claims 1 and 6, "A method of assaying for protease activity, comprising providing a nucleic acid construct" is unclear. Nucleic acid molecules are not protease substrates. The described construct would have to be translated and the encoded protein used as a substrate.

Claims 2-5 and 7, as dependent from Claims 1 and 6, respectively, are rejected for the same reasons. For purposes of examination, it is assumed that the nucleic acid construct is transfected into host cells for production of the encoded substrate.

In Claim 5, for the phrase "the protease is introduced" it is unclear whether the protease is being introduced into a reaction mixture in solution, into a cell, or into an organism. Clarification is required. For purposes of examination, it is assumed that the protease is being introduced into a cell.

In Claim 6, the phrase "expressing [of] the recombinant fluorescent substrate in the presence of a plurality of proteases has three possible interpretations. Either the substrate is expressed in a single cell comprising a plurality of proteases, the substrate is expressed in a plurality of cells wherein each cell has a single substrate, or both of the above. For purposes of examination, it is assumed that the substrate can be expressed in a single cell comprising a plurality of proteases or the substrate can be expressed in a plurality of cells, wherein each cell has a single protease.

Claim Rejections - 35 USC § 112-First Paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for the fluorescent protease substrate comprised of GFP1-157 fused to the N-terminus of a peptide having a substrate motif for the NS3/4A protease followed by GFP158-238, does not reasonably provide enablement for a method using any fluorescent protease

substrate comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Claims 1-3, 5, and 6 are so broad as to encompass a method using any fluorescent protease substrate comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. Claims 4 and 7 are so broad as to encompass a method using any fluorescent protease substrate comprised of an N-terminal portion of GFP fused to any protease substrate motif followed by the C-terminal portion of GFP. The scope of each of these claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of fluorescent reporters, protease substrate, and fusions thereof broadly encompassed by the claims. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired fluorescent protease substrate function requires a knowledge of and guidance with regard to which amino acids in the reporter and substrate sequences, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the polypeptides' structures relates to their function. However, in this case the disclosure is limited to the fluorescent protease substrate comprised of GFP1-157 fused to the N-terminus of a peptide having a substrate motif for the NS3/4A protease followed by GFP158-238.

While recombinant and mutagenesis techniques are known, it is not routine in the art to screen for multiple combinations of fusions proteins comprising a fluorescent reporter and a

protease substrates as well as multiple substitutions or multiple modifications thereof, as encompassed by the instant claims, and, furthermore, the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the results of such modifications are unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of Claims 1-3, 5, and 6, which are so broad as to encompass a method using any fluorescent protease substrate comprised of the N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. The specification does not support the broad scope of Claims 4 and 7, which are so broad as to encompass a method using any fluorescent protease substrate comprised of an N-terminal portion of GFP fused to any protease substrate motif followed by the C-terminal portion of GFP. The specification does not support the broad scope of Claims 1-7 because the specification does not establish: (A) all fluorescent reporters that can be used to prepare a fluorescent protease substrate comprised of the N-terminal portion of the reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter; (B) all protease substrate motifs that can be used to prepare a fluorescent protease substrate comprised of the N-terminal portion of a reporter fused to the motif followed by the C-terminal portion of the reporter; (C) all combinations of reporters and protease motifs that can be used in combination to produce a fluorescent protease substrate; (D) regions of the fluorescent substrate sequence which may be modified without effecting the function of the fusion protein as a

fluorescent substrate; (E) the general tolerance of the activity of fluorescent substrate to modification and extent of such tolerance; (F) a rational and predictable scheme for modifying any residues with an expectation of obtaining the desired biological function; and (G) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including methods of assaying protease activity using any number of fluorescent protease substrates comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of the identity of sequences having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claims 1-7 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

These claims are directed to methods of assaying protease activity using a genus of fluorescent substrates comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. The specification

teaches the structure of only a single representative species of such fluorescent substrates. Moreover, the specification fails to describe any other representative species by any identifying characteristics or properties other than the functionality of being a fluorescent substrate comprised of an N-terminal portion of any fluorescent reporter fused to any protease substrate motif followed by the C-terminal portion of the reporter. Given this lack of description of the structure of representative species encompassed by the genus of the claim, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that applicants were in possession of the claimed invention.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Anderson et al, 2001 (in IDS). Anderson et al teach a method for assaying protease activity using a fluorescent substrate (col 7, line 10), wherein the N-terminal portion of GFP is fused to a protease substrate followed by the C-terminal portion of GFP (col 2, lines 4-8). The fluorescent substrate can be used for detection of protease activity in a plurality of cells, i.e. a library of cells, expressing proteases (col 22, lines 51-56). Therefore, Claims 1-7 are rejected under 35 U.S.C. 102(b) as being anticipated by Anderson et al, 2001.

Examiner's note: the benefit of priority claimed for application US60/267,440 is not deemed valid, as the specification of said application does not describe the inventions examined here.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mahajan et al, 1999 (in IDS) in view of Abedi et al, 1998. Mahajan et al teach a method for assaying protease activity using a fluorescent substrate wherein cyan fluorescent protein (CFP) is fused to the N-terminus of a peptide substrate followed by GFP fused to the C-terminus of the peptide substrate. The fluorescent substrate is used for detection of protease activity in the presence a plurality of endogenous proteases (pg 405, parg 2/Fig 5 and pg 406, parg 2/Fig 6). Mahajan et al do not teach a method for assaying protease activity using a fluorescent substrate wherein, the N-terminal portion of a fluorescent reporter is fused to a peptide substrate followed by the C-terminal portion of the reporter. Abedi et al teach fluorescent peptides, wherein the N-terminal portion of GFP is fused to a peptide followed by the C-terminal portion of GFP. It would have been obvious to a person of ordinary skill in the art to use the method of Abedi et al to prepare a fluorescent substrate, wherein the N-terminal portion of GFP is fused to a protease substrate followed by the C-terminal portion of GFP. To do so is suggested by Abedi et al, wherein they teach that GFP can be used as a scaffold for the display of conformationally constrained peptides

(Abstract). One would be motivated to use a fluorescent substrate comprised of only GFP, rather than CFP plus GFP for two reasons. First, the polypeptide for the GRP-peptide substrate would be smaller and, therefore, less prone to random proteolysis. Second, one would want to avoid bleed-through background fluorescence, which would occur upon cleavage of the CFP-peptide-GFP fluorescent substrate to generate free CFP and GFP. The expectation of success is high, as fusions proteins in which a peptide has been inserted into GRP are known in the art (Abedi et al). Therefore, Claims 1-7 are rejected under 35 U.S.C. 103(a) as being unpatentable over Mahajan et al, 1999 in view of Abedi et al, 1998.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Sheridan L. Swope whose telephone number is 703-305-1696. The examiner can normally be reached on M-F; 9:30-7 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy can be reached on 703-308-3804. The fax phone numbers for the organization where this application or proceeding is assigned are 703-305-3014 for regular communications and 703-872-9307 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Sheridan L. Swope, Ph.D.

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